

EFFICACY REVIEW

Product: Maki Mini Blocks

Date: July 13, 2005

EPA File Symbol(s): 7173-239

DP Bar code(s): D313869

Chemical Code: Difethialone 128967

Formulation(s): Difethialone bait blocks

Purpose for Review: The purpose for this review is to determine if the two submitted studies support the revised basic, 3 alternate CSF's, and the addition of sewer and burrow use directions on the product label.

MRID(s): 464428-01 Hanson, J. M.. September 9, 2004. Difethialone Block 0111 (Weathered): Standard Rat Block/Pellet Laboratory Test 1.213 (12/2/90). Liphatech, Inc. Unpublished Report. Study No. 04011. 64p.

464216-01 Hanson, J.M.. September 9, 2004. Difethialone Block 0111 (Weathered): Standard Mouse Block/Pellet Laboratory Test 1.214 (12/2/90). Liphatech, Inc. Unpublished Report. Study No. 04029. 50p.

Good Laboratory Practices: Yes

Branch Supervisor: Meredith Laws, Branch Chief

Team Reviewer: John Hebert, Product Manager PM Team 07

IRB Reviewer: Geraldine R. McCann, Biologist

BACKGROUND: Liphatech, Inc. has applied to amend the current CSF's for Difethialone Block. The efficacy tests associated with these products were conducted according to the 40 CFR 160.35 (b) (6) (7), Good Laboratory Practices OPP Designation 1.213: Standard Norway Rat Anticoagulant Wax Block and Wax Pellet Laboratory Test Method and OPP Designation 1.214: Standard House Mouse Anticoagulant Wax Block and Wax Pellet Laboratory Test Method. This is a review of the efficacy tests submitted in support of the above named product.

REVIEW OF DATA:

1. 464428-01 Hanson, J. M.. September 9, 2004. Difethialone Block 0111 (Weathered): Standard Rat Block/Pellet Laboratory Test 1.213 (12/2/90). Liphatech, Inc.

DISCUSSION: This study was conducted to determine the efficacy of a weathered bait block (Difethialone Block 0111) formulated to control male and female Norway rats using a 15-Day, two-choice feeding test (OPP guideline 1.213 Standard Norway Rat Anticoagulant Wax Block and Wax Pellet Laboratory Test Method). A Certificate of Analysis was offered for the difethialone bait as Lot Number 17403 (manufactured on 06/23/2003) analyzed on 02/03/2004, at 23.49 mg/kg. The weathering log states it is the Log for Difethialone Block 0111 – Lot # 03004 – LTI #04011 and 04012. **There is no documentation to show that Difethialone Block 0111 bait (manufactured on 06/23/2003) was re-analyzed after the weathering process (documented from 02/07/2004 to 02/22/2004). The temperature records from the weathering chamber were not included, only the hand written log that is not complete (information from 4 days are missing = “No Observations”).**

Forty Wistar-Hannover rats arrived at the test facility on February 3, 2004, and were weighed on February 20, 2004 and again on March 15, 2004. The source of the animals is listed as “Charles River Laboratories 02/03/04”. The testing began February 23, 2004. The OPP guideline 1.213 was used as a protocol reference. The overall difference between the original average pretest weights for the male and female rats used in the test (for both groups, test and control) was 67.62 g. The maximum acceptable difference in average weights between sexes for laboratory rats is 50 g (1.213, 2.1). The Protocol Deviation 2 justification for the overweight rats was as follows: “These animals were ordered at weights (150-175 gram males/175-200 gram females) typically used to ensure staying within the 50 gram average weight difference range specified in the test method. This particular grouping of animals had a more widespread range of weights by the start of the study than anticipated, but because all animals still fell within the 150-300 gram range the study was commenced as scheduled.” There does not appear to be any reason for the males to have gained weight faster or more easily than the females, but I don’t have enough information to determine a solution. The 17+ grams should not affect the overall outcome of the study.

The rats were placed in individual, all-metal, mesh-bottom cages with a bottom surface area of 500 to 2000 cm². Glass jar feeders were used to issue the bait and diet. AIn order to provide the daily food requirement of 40 grams per animal per day minimum, a dosage of at least 40 grams of Difethialone Block 0111 (weathered) per cage through day fifteen was selected via free-choice oral feeding.” and Athe position of the jars in the cage was reversed on a daily basis.@ “The test substance was presented in the form in which it was manufactured for use and the form in which it was weathered. (ie: presented as whole weathered bait blocks and not altered or cut, shaven, or broken) by the personnel.”

The raw data to verify temperature and humidity in the testing facility was included with this submission on pages 59 to 63 of 64. The temperature ranged

from 66°F to 75°F and humidity ranged from 20% to 70%. The Dickson Circular Chart records were started February 9, 2004, and recorded constantly through March 15, 2004. The guidelines state (1.213, 5.1) the temperature must be kept between 20 to 25°C (68 to 77°F) and humidity between 50 to 55%. A protocol deviation was mentioned on page 44 of 64: "On page 3 of the protocol the temperature is stated to be 20 - 25°C and the relative humidity is stated to be 50-55%. The actual temperature range was from 18.9 to 23.9°C and the humidity range was 38 to 70% during the course of the study. Justification: What was reported is reasonable and should not affect the results of the test."

The guidelines specify the Standard OPP Rat and Mouse challenge diet to be prepared in a certain way. For these tests, the OPP diet has been labeled with A Lot Number 00604A, 00604B, and 04804A". Manufactured January 6, 2004, January 6, 2004, and February 19, 2004, with corresponding dates of analysis as follows: January 9, 2004, January 9, 2004, and February 19, 2004, respectfully.

As stated in the study report: "Each day the unconsumed test substance and challenge diet were collected from the cage and weighed. The quantity of each that was not consumed by the rats during the preceding 24 h was recorded and the amount consumed was calculated. Spilled test substance and challenge diet were recovered and weighed to establish exact consumption data. Where the spillage was damp or fouled by urine or feces, it was dried to approximately its original moisture content before weighing and then thrown out and replaced by fresh product. After the collection and weighing of unconsumed test substance and challenge diet the feed containers were replenished with fresh material. Unconsumed material was reused if it was still in its original form, still retained 50% of its original size, and was not fouled by urine or feces. **The test substance was not manipulated in any way (ie: not cut, shaved or broken) when replenishing the food containers.**" The weight of the spillage is not recorded in the raw data presented in the report. No raw data was included with this submission to show how the spillage was incorporated into the consumption equation.

Of the tested rats, only 2 males and 1 female gained weight (9.0 g, 12.9 g, and 10.5 g, respectfully) and. The 17 other tested rats lost weight (males = 2.7 g to 18.4 g and females = 1.7 g to 29.7 g). The control males and females all gained weight (5.9 g to 63.2 g). Overall mortality of the test animals was 100 %. Results of the rat test are summarized below:

Table 1. Rats on Difethialone Bait Blocks 0111 (weathered)
Pretest Weights Bait 15-Day Test-Consumption and Mortality

Sex	Average Group Weight (g)	OPP Diet Consumed (g)	Treated Bait Consumed (g)	Total Bait Consumption (g)
M (10)	65.37	1497.96	871.90	2369.86
F (10)	202.40	100% Mortality Days to death: 4-10 for males and 6-16 for females		Percent Treated Bait Consumed 36.79 %
Total (20)	62.97			

2. **464216-01** Hanson, J. M.. September 9, 2004. Difethialone Block 0111 (Weathered): Standard Mouse Block/Pellet Laboratory Test 1.214 (12/2/90). Liphatech, Inc. Unpublished Report. Study No. 04029. 50p.

DISCUSSION: This study was conducted to determine the efficacy of a weathered bait block (Difethialone Block 0111) formulated to control male and female C3H mice using a 15-Day, two-choice feeding test (OPP guideline 1.214 Standard House Mouse Anticoagulant Wax Block and Wax Pellet Laboratory Test Method.) Certificates of Analysis were offered for the Difethialone Block 0111 as Lot Number 13404 manufactured on 05/13/2004, analyzed on 05/18/2004, at 24.91 mg/kg. **A Certificate of Analysis for the weathered bait (Difethialone Block 0111 [Weathered]) was not included (or dated to reflect that it was done for the weathered baits) in this submission for verification.** The raw data to verify the temperature and humidity requirements for weathering the baits included on page 16 of 50 (dated May 24, 2004, to June 8, 2004) is missing 5 days of observations. The temperature is listed as being 37.7 to 37.8°C (99.86 to 100.04°F) and 92% to 95% humidity. Different stages of swelling are described progressively adding yellow, brown, white, and orange growth daily.

Forty C3H mice arrived at the test facility at an undetermined date and were weighed on June 8, 2004. The source of the animals is listed as CRL 5/26/2004 (Charles River Laboratories, Inc). The testing began June 9, 2004, using OPP guideline 1.214 as a protocol reference. The overall difference between the original average pretest weights for all mice used in the test (both male and female groups, test and control) was 1.90 g. The maximum acceptable difference in average weights between sexes for laboratory mice is 5g (1.214, 2.1). The mice were placed in single-sex groups of 10, in all-metal, solid-bottom cages with a bottom surface area of 2.16 ft². "Emptied soup cans were used to provide shelter." "The test substance and challenge diet were place in four glass jars each and presented alternately on two plastic trays to capture spillage." AIn order to

provide the daily food requirement of 10 grams per animal per day minimum, a dosage of at least 100 grams of Difethialone Block 0111(weathered) per cage per day through day fifteen was selected via free-choice oral feeding. The test substance was presented in the form on which it was manufactured for use and the form in which it was “weathered” (ie: presented as whole weathered bait blocks), and was not altered (ie: not cut, shaved, or broken) by the personnel. Challenge diet was also given in greater than or equal to the amount of test substance. Each day, the unconsumed test substance and challenge diet were collected from the cage and weighed. The quantity of each that was not consumed by mice during the preceding 24 h was recorded, and the amount consumed was calculated. Spilled test substance and challenge diet were recovered and weighed to establish exact consumption data.” **The weight of the spillage is not recorded in the raw data presented in the report. No raw data was included with this submission to show how the spillage was incorporated into the consumption equation.**

One Protocol Deviation was noted (page 32 of 50): “On page 3 of the protocol the temperature is stated to be 20 – 25°C and the relative humidity is stated to be 50 – 55%. The actual temperature range was 17.8 to 23.9°C and the relative humidity was 43 – 60% during the course of the study. The Justification states: “This is what actually occurred and it does not affect the outcome of the study.”

The raw data to verify the correct formulation of the OPP rat and mouse challenge diet was included with this submission and were labeled with Lot numbers 06504B and 13804A (pages 43 and 44 of 50). The Certificate of Analysis (pages 12 and 13 of 50) confirm the manufacturing date (03/05/2004 and 5/17/2004, respectfully) for both batches.

Of the tested females, all 10 lost weight (0.3 g to 4.8 g). Of the tested males, two gained weight (0.2 and 0.4 g). For the control animals, all gained weight (0.9 g to 7.6 g). Overall mortality of the test animals was 95%. Results of the mouse test are summarized below:

Table 2. Mice on Difethialone Bait Blocks (weathered)
Pretest Weights Bait 15-Day Test-Consumption and Mortality

Sex	Average Group Weight (g)	OPP Diet Consumed (g)	Treated Bait Consumed (g)	Total Bait Consumption (g)
M (10)	22.57	411.9	321.9	733.8
F (10)	20.69	95% Mortality Days to death: 4-15 for males and 4-11 for females		Percent Treated Bait Consumed 43.9%
Total (20)	1.88			

Efficacy Comments	<ol style="list-style-type: none"> 1. The raw data from the laboratory conditions for pretest, holding and testing conditions in the animal test facility have been provided for verification. 2. The raw data to verify the correct formulation of the OPP rat and mouse challenge diet and bait formulation were provided for verification 3. The weight of the spillage is not recorded in the raw data presented in the report. An explanation of spillage collection was offered in the report; however, no raw data was included with this submission to show the actual record of the spillage was incorporated into the consumption equation. 4. In future studies, documentation should always be included to show that the weathered bait (Difethialone Block 0111) was re-analyzed after the weathering process for both the rat and mouse tests and that no information is missing from the temperature/humidity records from the weathering chamber.
Conclusion(s)	The studies reviewed above are acceptable and support the addition of sewer and burrow use directions on the product label.
CSF Review	The new Basic CSF is acceptable and three (3) Alternate CSFs are acceptable.
Label Review:	In the sewer application instructions, add “block” to the phrase in the second sentence after “suspend bait”. The label is acceptable.